Restricted Water Intake Influences Male Reproduction in Two Strains of House Mice (*Mus musculus*)

RANDY J. NELSON

Departments of Psychology and Population Dynamics
The Johns Hopkins University, Baltimore, MD 21218

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NELSON, R. J. Restricted water intake influences male reproduction in two strains of house mice (*Mus musculus*). PHYSIOL BEHAV 43(2) 217–221, 1988.—Males from two strains of house mouse (*Mus musculus*) were subjected to ten weeks of simulated drought in the laboratory. Water availability was reduced to 50% of ad lib intake for 5 weeks, then further restricted to 25% of ad lib consumption for an additional 5 weeks. Individuals of the highly inbred (CF1) strain were generally unaffected by water restriction. Testicular and epididymal mass of restricted CF1 animals did not change relative to control mice with ad lib access to water. Seminal vesicle mass decreased in water restricted CF1 males, but spermatogenesis was not significantly influenced. Body mass was reduced 25.4% after water restriction. In contrast, male F1 progeny of wild-caught *Mus* substantially reduced reproductive organ mass after limited water intake. Spermatogenesis was significantly diminished, but no animals became completely aspermatic. Body mass declined 12.6% in water restricted wild-strain *Mus* as compared to animals with ad lib water availability These results are discussed in terms of their possible ecological significance.

**Seasonal breeding**  **Drought**  **Spermatogenesis**  **Male reproductive function**  **Body mass**

DOMESTICATED house mice (*Mus musculus*) are continuous breeders in the laboratory [6]. In nature, an apparent dichotomy exists between *Mus musculus* directly commensal with humans (i.e., mice that occupy dwellings, animal shelters, food storage facilities, etc.) and those animals that are noncommensal (i.e., mice that occupy grasslands and cultivated areas). Commensals have been reported to reproduce at all times of the year [1, 2, 6, 12, 24]. Noncommensals are often seasonal breeders (e.g., [5, 9, 13, 20–22]). Wild stocks of *Mus* breed continuously when housed in the laboratory [13]. The reproductive system of the male house mouse appears to be continuously “on” unless turned off by some extrinsic factor. The extrinsic factor(s) regulating *Mus* breeding remain(s) unidentified.

Extrinsic factors likely to influence *Mus* reproduction in nature include day length, temperature, and caloric and water availability. Photoperiod does not affect reproduction in house mice [6,23]. Although reduced caloric intake diminished reproductive competence in female *Mus*, moderate to severe food restriction does not inhibit sexual development in males [3,11]. However, cold temperatures and poor diet will suppress reproductive function in adult male mice [7,23]. Supplemental feeding of natural populations of seasonally breeding *Mus* induced winter breeding [9]. Lidicker [13,14] speculated that food and water availability could inhibit reproductive function in house mice. This proposition has been examined in field studies with mixed results (e.g., [4,21]). The hypothesis that restricted water availability could directly affect reproductive function was reexamined in the present laboratory study.

Previous work has demonstrated that a 50–75% reduction in water intake inhibits spermatogenesis in deer mice (*Peromyscus maniculatus*) independently from effects of food consumption, temperature or photoperiod [16,19]. It was suggested that water availability, per se, could serve as a potential cue for reproductive activities in deer mice. Phenotypic variation in spermatogenic responses was observed among deer mice in response to water restriction; sperm production ranged from normal to zero sperm numbers after 10 weeks of limited water intake [19]. Phenotypic variation in reproductive responsiveness to photoperiod, restricted food and temperature cues has also been observed in *Peromyscus* [10]. The phenotypic variants were considered to be specific morphs that were reproductively responsive to one or more environmental factors [10,19].

Two issues were addressed in the present study: (1) the role of water availability in the regulation of reproduction in *Mus* and (2) the genetic contribution to the variable reproductive responses to water restriction. True morphs [8,15] should differ genetically from one another; phenotypic variation in reproductive responsiveness to water restriction should be expected in wild-strain mice. Homogenic individuals should not display variation in reproductive responsiveness to simulated drought.

**METHOD**

**Animals**

Two strains of *Mus musculus* were used in the study. Male albino CF1 mice, obtained from an inbred colony, and
TABLE 1
MEAN (± S E M.) REPRODUCTIVE PARAMETERS OF TWO STRAINS OF Mus AFTER SUSTAINED WATER RESTRICTION

<table>
<thead>
<tr>
<th></th>
<th>Absolute Paired Testes Mass (mg)</th>
<th>Relative Paired Testes Mass (mg/100 g)</th>
<th>Paired Epididymal Mass (mg)</th>
<th>Seminal Vesicle Mass (mg)</th>
<th>Epididymal Sperm Number (×10⁷)</th>
<th>Testicular Sperm Number (×10⁷)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild Mus</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ad lib (n=15)</td>
<td>203.1 ± 5.68</td>
<td>43.2 ± 2.05</td>
<td>96.9 ± 4.18</td>
<td>151 6 ± 13.86</td>
<td>51 1 ± 3.82</td>
<td>38 9 ± 1.48</td>
</tr>
<tr>
<td>Restricted (n=42)</td>
<td>177.0 ± 3.38</td>
<td>33.1 ± 0.93</td>
<td>85 6 ± 2.51</td>
<td>112 9 ± 6.56</td>
<td>25.4 ± 1.61</td>
<td>25 0 ± 0.90</td>
</tr>
<tr>
<td><strong>CF1 Mus</strong></td>
<td></td>
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</tr>
<tr>
<td>Ad lib (n=14)</td>
<td>247.0 ± 4.90</td>
<td>96.35 ± 3.04</td>
<td>102.5 ± 3.10</td>
<td>269 7 ± 16.75</td>
<td>66.8 ± 3.71</td>
<td>36.7 ± 2.93</td>
</tr>
<tr>
<td>Restricted (n=49)</td>
<td>225.4 ± 4.36</td>
<td>66.14 ± 1.89</td>
<td>88 7 ± 1.60</td>
<td>175 3 ± 7.13</td>
<td>55 2 ± 1.96</td>
<td>34.0 ± 1.32</td>
</tr>
</tbody>
</table>

RESULTS

Testicular Function

Water restriction reduced relative, but not absolute, testicular mass in CF1 males (p<0.01). Reduced water consumption also reduced seminal vesicle mass (p<0.05), but did not affect epididymal mass or sperm numbers in this strain (Table 1).

Reduced water consumption in the wild-strain mice significantly reduced testicular, epididymal and seminal vesicle masses (p<0.01, p<0.05 and p<0.05, respectively) (Table 1). Epididymal and testicular sperm content was lower (p<0.05) in water restricted mice as compared to animals receiving ad lib water (Table 1). However, all animals possessed substantial sperm numbers; no aspermatic mice were discovered.

Food and Water Intake

Food consumption was unaffected by water restriction (p>0.05) (Figs. 1 and 2). For CF1 mice, food intake increased slightly after the onset of 50% water restriction, decreased after 75% restriction, then rebounded to elevated levels. Daily ad lib water consumption averaged 6.05±0.09 cc/day for CF1 mice. Both groups of wild-strain mice displayed large peaks of daily food intake at weeks 5 and 7 of the experiment. Examination of daily ambient temperature recordings, as well as other laboratory records failed to provide a cause for the twin peaks. Daily ad lib water consumption averaged 5.61±0.13 cc/day for wild-strain house mice.

Body Mass and Torpor

Body mass decreased 25.4% in water restricted CF1 mice (p<0.01) (Fig. 1). Restricted CF1 mice maintained body mass during the 50% rationing, but slowly lost mass during the four weeks of 75% water restriction. Body mass gradually decreased 12.6% after 10 weeks of water restriction in the wild-strain (p<0.05) (Fig. 2). There were no marked changes in body mass corresponding to the dramatic fluctuations of food intake. No mice exhibited torpor.

F₁ offspring of wild-trapped house mice, captured near Calgary, Alberta, Canada, were weaned at 21 days of age and housed separately in polypropylene cages (27.8×7.5 × 13 cm). Food (Wayne mouse breeder diet; Allied Mills, Inc., Chicago, IL) and tap water were continuously available. All house mice were maintained in colony rooms with 24 hr light:dark (LD) cycles (16 L:8 D; lights on 0600 hr CST) at 20±2°C with 50±10% relative humidity. Animals were used after 70 days of age or about 3-4 weeks after the onset of sexual maturity.

Food and Water Intake

Ad lib food and water consumption was recorded for 10 consecutive days for 65 and 60 male CF1 and wild-strain house mice, respectively. A randomly selected subset of 15 mice from each strain continued to receive ad lib food and water throughout the experiment. Fifty CF1 and 45 wild-strain Mus were allocated 50% of their individual ad lib water intake for 30 days. The mice were further restricted to 25% of their unrestricted water intake for an additional 40 days.

Body mass and twenty-four hour food consumption was determined weekly. Food intake was calculated as grams of food ingested/gram body mass; water intake was calculated as cc water/g body mass. All animals were checked twice daily for torpor.

Autopsy and Determination of Spermatogenesis

The masses of paired testes, epididymides and the seminal vesicles were recorded after autopsy. These data were analyzed as absolute values after correction for body mass.

Spermatogenic activity was determined. Paired testes (without capsule) and epididymides were minced and separately transferred quantitatively to an Eberbach blender and homogenized [19]. The number of sperm-shaped nuclei resistant to homogenization was determined in duplicate for each homogenate. The average was used to compute the final number of sperm per pair of testes or epididymides.

All data from each strain were subjected to analysis of variance (ANOVA) because the study was done in two parts. Mean values were considered statistically different if p<0.05.
FIG. 1. Mean (±S.E.M.) body mass (g) and 24 hr food consumption measurements (g) obtained weekly for male CF1 Mus musculus. Animals receiving ad lib water (n=15) are represented in the left panels and water restricted mice (n=50) are depicted in the right panels.

FIG. 2. Mean (±S.E.M.) body mass (g) and 24 hr food consumption measurements (g) obtained weekly for male wild-caught strain of Mus. Animals receiving ad lib water (n=15) are represented in the left panels and water restricted house mice (n=45) are depicted in the right panels.
DISCUSSION

Restricted water consumption did not affect absolute testicular or epididymal mass in the albino CF1 house mouse. Although seminal vesicle mass declined after water restriction, testosterone titers were adequate to maintain complete spermatogenesis in every individual of this inbred strain. Body mass declined substantially despite no significant changes in food consumption. In contrast, wild-strain *Mus* responded to restricted water intake with significant reductions in testicular, seminal vesicles and epididymal mass, as well as sperm production. No individual CF1 became aspermatic in response to limited water. Fertility was not assessed; however, these results suggest that water availability could affect reproductive function in nature.

The reproductive effects of reduced water consumption have been reported for a few rodent species. Total water deprivation reduced reproductive organ mass and litter production in gerbils [26]. Supplementation of water during the dry season in California voles (*Microtus californicus*) [14]: the effects of increased water consumption were not separated from the appearance of green vegetation in this study. Restricted water intake (~50%) caused gonadal regression in California voles [18], however, spermatogenesis was unaffected and fertility was not assessed. Supplementation of water during the dry summer non-breeding season also induced recrudescence and sperm production in *Peromyscus truei* [4]. Restricted water consumption induced gonadal regression among individuals of two subspecies of deer mice (*Peromyscus maniculatus*) (unpublished data). Phenotypic variation in reproductive responsiveness was evident in water-restricted deer mice with spermatogenic activity ranging from normal to no sperm production. Plasma testosterone levels decreased in all water-restricted animals, but plasma lutetianizing hormone (LH) titers were reduced only in aspermatic, water-restricted deer mice (unpublished data). Furthermore, fat content was reduced in aspermatic mice. It was suggested that differences in the initial body fat content or the hydrolysis rate of adipose tissue accounted for the differential reproductive responses to water restriction.

Differences in fat reserves could also account for the varied reproductive responses among *Mus* in the present study. The reduction in adipose tissue could directly suppress gonadotropin secretion [25]. Individuals of the wild-strain *Mus* are leaner than the domesticated CF1 counterparts. Presumably, male house mice could maintain their reproductive activities during mild droughts if they stored sufficient fat reserves and if they could locate fertile females in such conditions.

Plasma hormone levels were not directly measured in the present study. However, LH levels were sufficient to maintain some degree of spermatogenesis. Decreased seminal vesicle mass is consistent with reduced testosterone production. Further studies are required to assess the influence of water availability upon reproductive behavior, as well as on the fertility of oligospermatic house mice.

The degree of reproductive responsiveness displayed by water-restricted wild *Mus* is consistent with the characterization of this species as extremely opportunistic [6]. A complete cessation of spermatogenesis in response to drought conditions would require a minimum of 30 days after the onset of good conditions to reinstate sperm production. The maintenance of spermatogenesis at reduced levels insures a rapid response to improved conditions (e.g., [4,21]).

Individuals of the CF1 strain were reproductively unresponsive to decreased water consumption despite an average 24% reduction of body mass. Apparently, reproductive responsiveness to inhibitory environmental factors has been selected against during the process of laboratory domestication. The uniform lack of spermatogenic response in the CF1 males suggests that genetic factors account for the variation in reproductive responsiveness to environmental cues (e.g., [10,17,19]) and strengthens the argument that true reproductive morphs exist in other rodent species (e.g., [10,19]). It will require genetic analysis to ascertain the genetic contribution to this phenotypic trait. The preparation and study of the reciprocal crosses between the CR1 and the wild-strain mice would certainly be instructive. The present data support the hypothesis that wild *Mus* may use water availability to precisely time reproduction in nature. Reproductive organ mass, as well as sperm production, declined after water consumption was restricted. This reproductive response occurred in the absence of changing food intake, temperature or photoperiod. Further, reproductive responsiveness to water availability appears to be mediated genetically; spermatogenesis and gonadal size in the inbred CF1 house mice was invariably unaffected by reduced water intake.

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REFERENCES


